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Chapter 12

General summary and discussion

Summary and general discussion

Bacteraemia, the presence of bacteria in the bloodstream, was recognised more than a century ago [1]. At that time, 25mL of blood was cultured in liquid media and on agar plates [2]. If growth was observed, identification of the bacteria was achieved by determination of the phenotypic characteristics of the strain, including the presence or absence of haemolysis and the result of Gram-staining. Ever since, both the insight in clinical presentations that are associated with bacteraemia and the optimization of laboratory methods for detection of microorganisms from blood have evolved [3-5]. The ultimate goal of these developments is to permit the early institution of adequate treatment of the patient, which is important to improve prognosis, to reduce length of hospital stay and costs, and to prevent the development of antimicrobial resistance [6-13]. The aim of this thesis was to study strategies for improvement of diagnosis and management of bloodstream infections (BSI) at different stages of the clinical and diagnostic process (figure 13). These include the design of models for bedside prediction of bacteraemia, the speed-up of time to identification and susceptibility testing of microorganisms in growth-positive blood cultures by fluorescence in situ hybridisation (FISH) and direct E-test, and the use of quantitative polymerase chain reaction (PCR) assays for the direct detection of bacterial DNA in blood, without prior cultivation. In general, clinical assessment of the patient on presentation will result in initial therapeutic decisions. The combination of risk factors, presenting signs and symptoms, and results of laboratory tests will lead to an estimation of the likelihood of BSI. Dependent on this, empirical antimicrobial treatment will be initiated, and the choice of and mode of administration of antibiotic may be different when BSI is suspected than in case a localised infection is more likely, e.g. the use of broad spectrum antibiotics or intravenous rather than oral administration of antimicrobial agents may be more opportune. The impact of identifying clinical predictors of BSI to support these therapeutic decisions is high in settings where resources for laboratory and radiological tests are low. The Queen Elizabeth Central Hospital (QECH) in Malawi, a country in sub-Saharan Africa, is a good example of a setting that would benefit from good clinical predictors of BSI. Almost one million people in this country are infected with the human immunodeficiency virus (HIV), an important risk factor for BSI [14-16]. Microbiological and radiological facilities are limited and patients

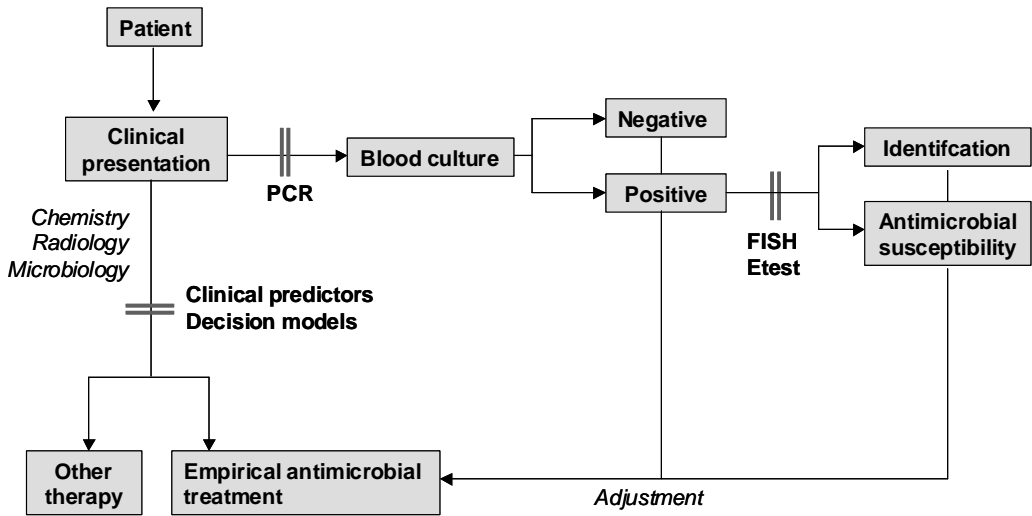


Figure 13. Flow-chart with the strategies that are evaluated in this thesis for diagnosis and management of bloodstream infection. The different strategies are marked with a double bar. PCR, polymerase chain reaction; FISH, fluorescence in situ hybridization.

are usually treated empirically. Therefore, a study was carried out at QECH to determine clinical predictors of BSI as described in **chapters 2 and 3**.

BSI was present in approximately one third of the febrile adult patients on admission to the medical wards. The most prevalent microorganisms isolated from blood cultures were non-typhoid *salmonellae* (NTS), *Streptococcus pneumoniae* and *Mycobacterium tuberculosis*. The majority of patients with NTS bacteraemia did not have a clear clinical focus of infection, whereas *S. pneumoniae* bacteraemia usually presented secondary to a local infection. The strong predictive value of splenomegaly and body temperature higher than 39°C for NTS bacteraemia is an important observation that could aid in the management of HIV-infected febrile patients in a resource-poor setting. We found a high prevalence of *M. tuberculosis* bacteraemia in our study. Several authors have previously advocated the routine use of mycobacterial blood cultures to diagnose *M. tuberculosis* bacteraemia in countries with a high prevalence of HIV infection and tuberculosis (TB), including Malawi, because of the high mortality associated with untreated mycobacteraemia [17,18]. However, mycobacterial blood cultures are expensive and their clinical impact may be limited because patients might be dead or discharged from hospital by the

time the results are available [19-22]. In this context, clinical predictors and routinely microbiological methods for detection of TB can be most helpful to establish the diagnosis of *M. tuberculosis* bacteraemia, as described in **chapter 3**. Clinical indicators of *M. tuberculosis* bacteraemia were cough, chronic fever, severe anaemia and HIV seropositivity. TB was diagnosed with routinely available investigations (sputum-smear, chest X-ray, fine needle aspiration) in the vast majority of patients with *M. tuberculosis* bacteraemia. Thus, *M. tuberculosis* bacteraemia in this setting usually presents with a detectable focus of infection rather than that it is primarily an intravascular infection, as suggested by others [18,19,23]. However, additional studies are warranted to further characterise those patients without a clear focus of TB but with *M. tuberculosis* bacteraemia. An important observation in this regard was the high prevalence of *M. tuberculosis* bacteraemia in patients that were eligible for a diagnostic trial of TB treatment. This suggests that the diagnostic value of this trial lies more in its indications than in the trial itself. Therefore, although only a small number of patients were included in our study, a trial of TB treatment does not seem to add to the diagnosis of TB. Instead, it seems reasonable to treat these patients directly with short-course chemotherapy, and clinical validation of this approach in a larger cohort is warranted.

The routine availability of blood cultures to detect bacteraemia or mycobacteraemia does not seem feasible in a resource-poor setting like QECH in Malawi. Instead of putting money and effort in creating such services, it would be better to focus on the improvement of clinical education and strengthening of basic laboratory facilities. Nevertheless, the intermittent evaluation of blood culture isolates at a few hospital settings is important to provide and monitor the epidemiological spectrum of microorganisms causing BSI. These epidemiological data will help to determine the choice of empirical antimicrobial treatment in settings without blood culture facilities. At present, various programmes have emerged to supply highly active antiretroviral therapy (HAART) to HIV infected patients in Malawi [24]. HAART was not yet routinely available at the time our study was performed. The widespread treatment of HIV-infection may change the incidence, spectrum and presentation of severe bacterial infections, including BSI [25,26]. This necessitates a re-evaluation of clinical predictors of BSI once the majority of HIV-infected patients have been enrolled in the antiretroviral therapy (ART) programmes.

We used multiple logistic regression (MLR) analysis to identify clinical predictors of BSI in adult patients admitted to QECH. However, although these models provide insight in the predictive value of individual factors for BSI, translation into clinical practice may be difficult. An alternative approach is the use of risk stratification models or the design of a decision tree with classification and regression tree (CART) analysis as described in **chapter 4** [27-29]. The decision tree that we designed for prediction of BSI in febrile medical patients had a reasonable accuracy and better performance than the MLR model. Peak temperature, nadir albumin and nadir thrombocyte count were predictors in both models, but procalcitonin (PCT) concentration was only included in the decision tree. In fact, PCT was the principal discriminator in the decision tree, which shows the model-dependency of predictors. The observed strong discriminative value of PCT is in accordance with other studies that show that PCT may be a more specific marker of BSI than C-reactive protein (CRP) or white blood cell count (WBC) [30-34].

At the time the data from the Malawi cohort were analysed, the programme for CART analysis was not available to us. If CART analysis had been performed on those data, body temperature and splenomegaly would have been the main discriminators between patients with and without bacteraemia (RPH Peters, personal observation). Also, no prospective validation was performed of the prediction models described in **chapters 2, 3 and 4** due to the low number of patients with BSI. Other studies show that the reproducibility of models may be different in other settings or patient populations [35,36]. As such, prospective confirmation of the MLR and CART models for prediction of BSI is crucial to define their potential clinical applicability. An important facilitator in this regard is the emerging use of handheld computers and electronic patient files. Decision models can be installed on these computers and might be used for an automatically calculation of risk of BSI in the individual patient. Although the use of such programmes may be highly supportive when making decisions on empirical treatment, computer-calculated predictions should never be used as a substitute for critical thinking or responsibility by the clinician.

The blood culture is the gold diagnostic standard for the diagnosis of BSI. However, the impact of blood culture results on clinical management may be limited due to the relatively long turnaround time, a low sensitivity for

fastidious microorganisms, and the reduced yield when cultures are obtained under antimicrobial treatment [37-40]. As a result, the majority of therapeutic decisions related to BSI are taken at the moment that blood is drawn for culture and when Gram-stain characteristics of growth-positive blood cultures become available [41]. Molecular assays including FISH and PCR can be used to speed-up time to identification of microorganisms from growth-positive blood cultures [42,43]. In **chapter 5**, the routine use of FISH for identification of microorganisms from growth-positive blood cultures is evaluated, with emphasis on time to diagnosis by FISH compared to the routine identification methods. The majority of microorganisms, especially Gram-positives, could be identified with FISH within 3-4 hours. This number could be further increased with the inclusion of extra probes in the assay, for example probes targeting rRNA of *Enterobacter cloacae* or *Proteus mirabilis*. Although the time-gain to identification by FISH compared to preliminary and final culture identification was quite significant, it is questionable whether FISH will be used in routine practice, especially in settings where preliminary culture identification is performed. The main reason is that clinicians might not await FISH results for another 3-4 hours after notification of Gram-stain results before initiation or adjustment of empirical antimicrobial treatment. Therefore, the standard FISH procedure was modified as described in **chapter 6** to reduce turnaround time to less than one hour. This modified FISH assay had a similar performance as the standard FISH assay. With FISH results available within one hour after Gram-stain, clinicians may await FISH results before initiation or adjustment of antimicrobial therapy.

As shown in **figure 7**, identification of microorganisms with FISH can have clear implications for the antimicrobial management of the patient. Recently, it was demonstrated that therapeutic interventions based on identification of microorganisms in growth-positive blood cultures by FISH reduce antibiotic usage, length of stay and hospital costs [44-46]. In these studies, FISH is used to discriminate between *Candida albicans* and non-albicans *Candida species* and to differentiate between *S. aureus* and coagulase-negative staphylococci. However, these studies were carried out at settings in the United States where no preliminary identification is performed. It is unclear whether the observed reduction in antibiotic usage after FISH identification could also have been achieved by implementation of preliminary culture identification, but at a lower cost. Furthermore, the

studies involve two clinical conditions where clear therapeutic consequences are associated with the FISH result. Although they provide a first step in the assessment of cost-effectiveness of FISH for identification of microorganisms in growth-positive blood cultures, a randomised prospective study, preferably in multi-center format, is highly warranted to assess the clinical impact, feasibility and cost-effectiveness of FISH in routine practice.

Identification of microorganisms in growth-positive blood cultures by FISH or PCR can lead to the adjustment of antimicrobial treatment. The choice for a change of antibiotics is based on the epidemiologically most likely susceptibility pattern of the microorganism that was identified by FISH or PCR. Determination of the antimicrobial susceptibility of the isolates is routinely done with disk-diffusion tests or with the automated VITEK system. An E-test performed directly on positive blood cultures, prior to species identification, may provide an alternative approach for standard susceptibility testing; the timely availability of the test result might also decrease antibiotic consumption [47]. As shown in **chapter 7**, direct E-test guided by FISH results can be used to obtain antimicrobial susceptibility data of microorganisms from growth-positive blood cultures within 24 hours. Direct E-test in general provides a similar classification of antimicrobial resistance as disk-diffusion or VITEK, although a more conservative result was generated in a few cases. As a result, only a few patients would have been treated with other antibiotics on basis of direct E-test than based upon the results of disk diffusion or VITEK, but always with antibiotics for which the microorganism is susceptible. However, the cost-effectiveness of direct E-test is highly questionable and needs to be investigated.

Although FISH offers faster identification of microorganisms from growth-positive blood cultures than routine identification techniques, the initial, time-consuming step of culturing blood has still to be performed. This can be overcome by the direct detection of microorganisms in blood by PCR, without prior cultivation. The use of PCR assays for detection of bacteraemia offers a faster alternative to culturing blood and is presumably not affected by the prior use of antibiotics [48,49]. **Chapters 9 and 10** are confined to the detection of *Staphylococcus aureus* and *Enterococcus faecalis* DNA in blood by quantitative PCR (QPCR) during bacteraemia in critically ill patients, while **chapter 11** describes the clinical relevance of detection of *Streptococcus pneumoniae* DNA in blood samples from

patients suspected of community-acquired pneumonia or meningitis. The sensitivity of these three QPCR assays compared to blood culture outcome lies in the range of 70-80%. This apparently low sensitivity may be explained, at least in part, by the small volume of blood (200 μ L) that was tested compared to the larger volume of blood inoculated into blood culture bottles (10mL), which may result in a sampling error. As such, the current technical developments are aimed at increasing the volume of blood that can be processed in DNA isolation, but the consequent inhibitory effects on PCR have to be overcome [50-52].

The specificity of the QPCR assays for detection of BSI in whole blood samples ranged from 92-96%. However, it is highly questionable whether positive PCR results in the absence of growth-positive blood cultures clinically should be considered 'false-positive'. The majority of patients with positive PCR reactions for *S. aureus*, *E. faecalis* and *S. pneumoniae* and not growth in simultaneous blood cultures had a clinically manifest focus of infection with the respective microorganism, as demonstrated by the isolation of the pathogen from other body sites than blood. In addition, a considerable number of those blood samples had been obtained after the initiation of antimicrobial therapy which reduces the yield of blood cultures considerably, but presumably has no effect on PCR [39,49]. It is not possible to determine whether these positive PCR reactions are related to true, undetected bacteraemia, represent circulating dead bacteria, or merely originate from DNA leakage from a local infection into the bloodstream, or even from colonization. Instead of 'bacteraemia', it might be better to use the term 'DNAemia' in the context of QPCRs on blood, because this does not commit to the origin of DNA or viability of bacteria in the bloodstream. Future studies should be focused on increasing our understanding of the clinical relevance of such a 'DNAemia'. Possible approaches are the determination of the bacterial DNA load (BDL) in blood as a quantitative marker of DNAemia to distinguish infection from colonization, or the comparison between DNA and RNA content as a measure of viability [53].

Results of PCR assays directly on blood samples may be available a few hours after the blood sample was obtained and might influence the institution of antimicrobial therapy. For example, a positive *S. aureus* PCR in blood from a patient suspected of endocarditis would establish the diagnosis and result in a more directed treatment at an early stage.

However, in clinical practice, many microorganism-specific PCR assays have to be performed to cover all possible microorganisms involved in the patient's condition. Instead, PCR algorithms may be used, if possible guided by the panel of microorganisms involved in the patient's condition, which has become more and more feasible with the decreasing turnaround time of PCR amplification systems [43].

Instead of using microorganism-specific PCR assays, a broad-range, universal PCR assay may be used that theoretically detects all bacterial or fungal DNA in one reaction. These assays are usually based on the detection of the highly conserved 16S gene of bacteria (eubacterial) or the 18S gene or internal transcribed spacer (ITS) regions of fungi (panfungal) [54-56]. These assays have been used for rapid prediction of BSI in patients presenting to the emergency department, neutropenic patients and neonates [57-59]. The performance of these broad-range PCR assays is variable, but may be better in blood samples from neonates (sensitivity 96-100% and specificity 58 to 98%) than in blood samples from adult patients (54-100% and 93-99%) [57-64]. Although there is no obvious reason for the difference in performance of these assays, a possible explanation could be that the concentration of microorganisms in blood of neonates is higher than in adults. A positive PCR amplification signal with the broad-range PCR does not specify from which specific microorganism DNA is present in the bloodstream. For that purpose, sequence-analysis of the broad-range amplification product is required [65]. However, the routine performance of sequence analysis is not feasible because of the high costs and relatively long turnaround time. Another disadvantage of these broad-range PCR assays is that not only clinically relevant, but also contaminating DNA is detected. This contaminating DNA can originate from many sources, including the commercially available kits that are used for DNA isolation [66,67]. Misinterpretation of false-positive results due to contamination potentially has adverse clinical consequences, as described in **chapter 8**. Therefore, efforts should be made to reduce contamination in diagnostic kits, especially for DNA isolation. While much progress has been made with the introduction of 'closed', real-time systems for PCR amplification detection, the developments to reduce contamination during DNA isolation have been scarce. Improvement of this situation is critical for future application of the broad-range assays in routine diagnostic practice. All taken together, the clinical role of broad-range PCR assays in the diagnosis

of BSI may be limited to establishing a diagnosis in patients with ongoing unknown illness despite other microbiological tests, e.g. persisting unexplained fever. When there is overt clinical infection, the direct use of an algorithm or panel of species-specific PCR assays seems to offer a better alternative.

The BDL in blood offers a quantitative measure of bacteraemia and is discussed in **chapters 10 and 11**. The receiver operating characteristic (ROC) curves generated for *S. aureus*, *E. faecalis* and *S. pneumoniae* BDL suggest that prediction BDL may be a better and certainly more specific marker of bacteraemia than the routinely used inflammatory markers CRP and WBC. With a further decreasing turnaround time of PCR assays, it will be feasible to obtain blood samples for determination of BDL simultaneously to full blood count and chemistry, and use the results at the same point in time of the diagnostic process.

BDL correlated positively with levels of CRP in critically ill patients with positive PCR amplification results, while the correlation of *S. pneumoniae* BDL with CRP was manifest for the entire cohort of patients suspected of community-acquired pneumonia or meningitis. The different correlations in these cohorts may be explained by the much more prominent role of *S. pneumoniae* as a pathogen involved in bacterial respiratory tract infections, as compared to the diverse aetiology of illnesses for which blood cultures are indicated in the intensive care unit (ICU). As such, the increase of CRP levels may be the result of other illnesses in a higher proportion of critically ill patients than in patients with community-acquired respiratory tract infections. On the other hand, due to the strict monitoring of patients in the ICU, blood samples may have been obtained at a different stage of the illness than those from patients with community-acquired episodes. As such CRP levels may be low despite the presence of BSI, because of the relatively slow response of CRP to infection. Both explanations are also reflected in the low discriminatory value of WBC and CRP that was observed in the ICU cohort compared to the strong discriminative value of those variables in the other cohort suggesting a more heterogeneous patient population in the ICU. Nevertheless, the observed positive association of BDL with CRP suggests that BDL is related to the degree of inflammation, with a higher BDL evoking a stronger inflammatory response. This idea is supported by associations that were observed for BDL and

microbiological invasiveness and clinical severity of infection, and the higher *S. pneumoniae* BDL in patients who fulfilled the systemic inflammatory response syndrome (SIRS) criteria on admission. Despite these associations, the relation of BDL with clinical symptoms, severity of illness and prognosis has yet to be elucidated. The anecdotic data from one patient as depicted in **figure 10** suggest that the course of *S. aureus* BDL may mimic the course of temperature during bacteraemia, although no general conclusions should be drawn from this case report. In contrast to our data, BDL on admission correlated with disease severity and short-term outcome in patients with systemic meningococcal disease [68,69]. However, the average values of meningococcal BDL in blood during meningococcaemia are much higher and more diverse than the BDLs observed in our cohorts, possibly associated with the more fulminant nature of that condition. Therefore, the numbers of patients with BSI in our cohorts may have been too low to detect associations between BDL and disease severity or prognosis.

The strategies to diagnose and manage BSI have gradually improved during the last century. With the recent emergence of molecular tests, the first steps are made towards a rigorous change in diagnostics of BSI which will have even more effect on clinical practice than the introduction of automated detection systems in the 1960s. The short-term developments to improve the diagnosis of BSI are aimed at the rapid identification of microorganisms in growth-positive blood cultures by FISH assays or PCR algorithms. Although these are promising tools to speed-up the identification of the causative microorganism in BSI, it is still essential to perform a time-consuming culture step prior to FISH or PCR. Therefore, for the long term, it is to be expected that the direct quantitative molecular detection of circulating bacteria and bacterial DNA fragments in blood will become the standard for diagnosing BSI. To achieve this goal, the sensitivity and specificity of the current QPCR assays have to be further improved, the risk of contamination reduced, and the turnaround time should even be further decreased. In addition, with the increasing understanding and opening up of the bacterial genomes and in combination with the mounting knowledge of antimicrobial resistance genes and mechanisms, molecular assays for determination of antimicrobial susceptibility will be more and more incorporated in the routine diagnostic

process. For example, a recent study shows that DNA microarray can be used for simultaneous identification and testing of genotypic antimicrobial resistance of *S. aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* [70]. The integration of molecular assays for identification and determination of antimicrobial resistance will be important spearheads in the future technical developments. The clinical interpretation of the DNAemia detected by these molecular assays will evolve with the increasing knowledge of the relationship between BDL and clinical and microbiological characteristics. The potential theoretical value of monitoring BDL is appealing and warrants further investigation of the kinetics of DNA in blood. Possibly, the course of BDL can be used to predict prognosis, to monitor response to treatment, or as a surrogate marker in clinical trials to evaluate efficacy and duration of antimicrobial treatment in bacteraemic conditions such as endocarditis.

The individual patient is eventually the one who has to benefit from all these technical developments, ultimately by receiving adequate antimicrobial treatment at the earliest possible moment. BDL provides another piece of the clinical jigsaw that lays out to the clinician when making bedside decisions about empirical therapy. As such, the clinical management of patients suspected of BSI will always remain an integrated assessment of risk factors, clinical symptoms and laboratory factors, which can only be supported but never replaced by high-tech laboratory facilities.

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